Investigations into the Control of Litter Size in Swine: II. Comparisons of Morphological and Functional Embryonic Diversity Between Chinese and American Breeds[1,2,3]

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ABSTRACT: It has been suggested previously that the increased litter size of the prolific Chinese Meishan pig may result from an increased littermate embryonic synchrony. This study compared embryonic diversity in Meishan and domestic (white line crossbred) sows. Third-parity Meishan sows (n = 14) and second-parity domestic sows (n = 15) were observed for estrus every 6 h and were hand-mated at 24 and 30 h after first observed estrus (d 0) to boars of the same breed. The sows were slaughtered on d 11.9 ± .1 (mean ± SEM). Embryos were flushed from each uterine horn and were individually sonicated, lyophilized, and frozen (−86°C) until they were assayed for estradiol-17β ($E_2\beta$), DNA, and protein content. Ovulation rate was higher ($P < .001$) in Meishan (24.9 ± 1.1) than in domestic sows (15.2 ± .7). The average littermate embryonic diameter was smaller ($P < .001$) for Meishan than for domestic sows, although morphological embryonic diversity (standard deviation for diameter) did not differ ($P > .10$) between the two breeds. In addition, for embryos of the same diameter, no differences ($P > .10$) in number of embryonic cells (micrograms of DNA/embryo) or in cell size (protein:DNA ratio) were observed for either breed. The more advanced (> 6 mm) embryos from Meishan sows contained less ($P < .001$) $E_2\beta$ (picograms/embryo) than did embryos of the same size from domestic sows. The prolificacy of the Meishan sow may result from both an increased ovulation rate and reduced $E_2\beta$ production by Meishan embryos. The reduced $E_2\beta$ production by Meishan embryos may result in more gradual alterations in uterine environment with an increased survival of less-developed littermates.

Key Words: Meishan, Pigs, Embryo, Diversity, Size, Estrogens

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Introduction

As porcine embryos develop from spherical to filamentous forms, embryonic estrogen production increases (Pusateri et al., 1990), resulting in changes in uterine histotroph composition (Geisert et al., 1982). Estrogen production by the more advanced embryos in a litter may alter the uterine environment, resulting in the death of less-developed littermate embryos (Pope and First, 1985; Pope et al., 1990).

Meishan gilts and sows are more prolific than either American or European breeds, averaging three to five more pigs per litter (Cheng, 1983; Bolet et al., 1986; Haley et al., 1990). The increased prolificacy of Meishan gilts vs that of gilts of domestic swine breeds has been attributed to differences in either preimplantation embryonic developmental rate (Bazer et al., 1988; Youngs et al., 1993) or ovulation rate (Ashworth et al., 1990; Haley et al., 1990; Wilmut et al., 1992). However, due to marked differences in both the age at puberty (Bolet et al., 1986) and the degree of ovulation rate increase with advancing age (Cheng, 1983) between Meishan and domestic gilts, the specific factor(s) resulting in breed differences in prolificacy remain unclear.

In light of the contradictory reports on the specific factors that influence prolificacy, we compared ovulation rate and morphological (diameter, cell number,

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[3]Mention of names is necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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cell size) and functional (estrogen content) diversity of littermate embryos of Meishan and domestic (white crossbred) sows with documented differences in litter size.

Materials and Methods

Animals

Fourteen third-parity Meishan sows and 15 contemporary, second-parity, domestic sows derived from a crossbred foundation with equal (1/4) contribution from Yorkshire, Landrace, Large White, and Chester White breeds were used. A description of genetic background for Meishan sows has been reported (Young, 1992). The experiment was conducted jointly by the Roman L. Hruska U.S. Meat Animal Research Center ARS, USDA, Clay Center, NE, and Iowa State University, Ames.

Meishan and domestic sows were separated from their litters after a 28- to 32-d lactation period and housed within breed in adjacent pens (three to eight sows per pen) in a totally enclosed confinement building. Sows were checked daily for postweaning estrus and then, beginning on d 17 of the first estrous cycle, sows were checked for estrus every 6 h with an intact, sexually experienced boar until the cessation of estrus. Sows were hand-mated at 24 and 30 h after the onset of estrus (d 0) with a boar of the same breed. The interval from onset of estrus to slaughter was calculated to the nearest 6 h plus 3 h to correct for the midpoint between the last observation at which estrus was not observed and the first observation of estrus for each sow. The interval is reported as the day of gestation and averaged 11.9 ± .1 d for Meishan and crossbred sows. At slaughter, the reproductive tract was removed immediately after stunning and exsanguination. The broad ligament was trimmed from each uterine horn and the number of corpora lutea per ovary was recorded after ovarian dissection.

Embryo Collection

The trimmed uterus was cannulated by inserting and suturing a glass cannula (10 mm o.d.) through a small incision made in the uterine wall 2 to 3 cm from the utero-tubal junction on each uterine horn. Uterine forceps were clamped proximal to the cervix and on the contralateral uterine horn proximal to the uterine body before each uterine horn was flushed by infusing 20 mL of phosphate buffered saline (**PBS**; 33 m*M* NaH_2PO_4, 35 m*M* Na_2PO_4, 125.9 m*M* NaCl; pH = 7.4) into the uterine body with a blunt, 18-gauge needle. Flushings were collected into a sterile glass Petri dish and immediately placed on ice. The number of embryos was recorded and individual diameters determined to the nearest .125 mm using an ocular micrometer mounted in a stereomicroscope. Each embryo was rinsed with fresh 4°C PBS and then centrifuged (4,000 × *g* for 2 min) to remove excess PBS. The embryos were lyophilized, reconstituted in 1 mL of PBS with 2 *M* NaCl and 2 m*M* EDTA, sonicated for .5 min, and frozen (−86°C) before transport to Iowa State University, Ames, for determination of estradiol-17β (**$E_2\beta$**), DNA, and protein content. Uterine flushings were also collected and frozen at −20°C until they were analyzed for $E_2\beta$ concentration.

Assay Methodology

Estrogen. Embryonic estrogen content was quantified in unextracted samples by methods previously reported and validated (Kesler et al., 1977; Redmer and Day, 1981; Christenson et al., 1984), using the same fully characterized antibody (Lily #030073 $E_2\beta$ antiserum). Precision and accuracy of the assay were determined by addition of 5, 25, and 100 pg of $E_2\beta$ to an embryo pool and resulted in recoveries of 5.6, 27.1, and 117.1 pg, respectively. The sensitivity of the assay, as determined by the amount of steroid yielding 95% of the counts in buffer control tubes, was 2 pg/tube. Multiple aliquots of a pool of embryonic tissue were included in all six assays, resulting in inter- and intraassay CV of 14.9 and 3.8%, respectively. The $E_2\beta$ content of uterine flushings was determined by the same $E_2\beta$ assay. However, uterine flushings (1 mL) were extracted once in 5 mL of diethyl ether (HPLC grade; average percentage recovery > 90%) before assaying. All samples were run in a single assay, and the intraassay CV was 17.1%.

DNA. Embryonic DNA content was determined using the method of Labarca and Paigen (1980) as modified by Pusateri et al. (1990). Calf thymus DNA was sonicated and serially diluted for a standard curve of 1.0, .75, .5, .125, .025, .005, and 0 μg/mL. Several aliquots of a pool of pig splenocytes were included in each assay to determine assay variation. Equal aliquots of tissue homogenates or standards were combined with DNA specific dye Hoechst 33258 (Calbiochem, San Diego, CA). Fluorescence was measured at both excitation (358 nm) and emission (456 nm) wavelengths with a Gilford Fluoro IV Spectrofluorometer. The sensitivity of the assay was .005 μg of DNA/mL. The inter- and intraassay CV were 6.1 and 3.2%, respectively.

Protein. Protein content in embryonic tissue was determined using the Bio-Rad protein assay (Bio-Rad Chemical Division, Richmond, CA). The standard curve consisted of serial dilutions of bovine serum albumin (crystalline; Intergen Company, Purchase, NY) at 8.0, 6.0, 4.0, 2.0, 1.0, .5, .3, and 0 μg/mL. Absorbance was measured on a Gilford spectrophotometer at a wavelength of 595 nm. The sensitivity of the assay was 1 μg of protein/mL. The inter- and intraassay CV were 7.7 and 6.4%, respectively.

Table 1. Ovulation rate and embryo recovery for Meishan and domestic sows

Breed	n	Ovulation rate	No. of embryos recovered per sow	Percentage of recovery (no. of embryo/no. of CL)
Meishan	14	29.9 ± 1.1[b]	22.4 ± 1.2[b]	93 ± 2[a]
Domestic	15	15.2 ± .7[a]	14.7 ± 1.1[a]	96 ± 3[a]

[a,b]Means ± SEM within a column with different superscripts differ ($P < .001$).

Statistical Analysis

Data were analyzed using the GLM procedure of SAS (1985). Individual embryo data were pooled by sow to examine the differences in littermate embryonic diversity.

Dependent variables analyzed included ovulation rate, number of embryos recovered, embryo recovery rate, average embryonic diameter (millimeters), DNA content (micrograms/embryo), protein content (micrograms/embryo), the protein:DNA ratio, and $E_2\beta$ content (picograms/embryo). The independent variable was breed. Data from eight sows that were included in analyses of ovulation rate and embryo recovery were excluded from analyses of embryonic data. Five of these sows were excluded from the embryo analyses because of either the presence of filamentous embryos (two Meishan and two domestic sows) or extremely low embryo recovery (35%; one domestic sow). All the embryos used in this study were spherical and ≤ 10 mm in diameter. No transitional-stage embryos (i.e., ovoid or tubular) were recovered from any gilt. Embryos from the remaining three sows (one Meishan, two domestic) were used in a separate experiment and were not available. Breed differences in uterine flushings content of $E_2\beta$ were also analyzed using the GLM procedure.

Results

At the second postweaning estrus, ovulation rate was greater ($P < .001$) for Meishan than for domestic sows (Table 1). Although the percentage of recovered embryos was similar ($P > .10$) for both breeds, Meishan sows had a greater ($P < .001$) number of embryos recovered per female than did domestic sows.

The average embryonic diameter (millimeters) within a litter was smaller ($P < .001$) for Meishan than for domestic sows (Table 2). Within-litter standard deviation of embryo diameter indicated no difference ($P > .10$) in embryonic diversity between Meishan and domestic sows. Further, no difference in the range of embryo diameters was observed in either breed (Figure 1).

Because embryonic diameter and embryonic DNA content (micrograms/embryo) were highly correlated ($r = .996$; $P < .001$), the average DNA content of littermate embryos from Meishan sows was lower ($P < .001$) than that of littermate embryos from domestic sows (Table 3). However, the average protein:DNA ratio, used as an indication of cell size (Baserga, 1985), for littermate embryos was similar ($P > .10$) between breeds.

The average littermate embryonic $E_2\beta$ content (picograms/embryo) within a litter was lower ($P < .001$) for Meishan than for domestic sows (Table 4).

Table 2. Average littermate embryonic diameter and within-litter embryonic diversity for Meishan and domestic sows

Breed	n[a]	Embryonic diameters, mm: Mean ± SEM	Embryonic diameters, mm: SD
Meishan	11	4.3 ± .4[b]	1.4[b]
Domestic	10	6.9 ± .3[c]	1.1[b]

[a]Excludes data from eight sows.
[b,c]Means ± SEM within a column with different superscripts differ ($P < .001$).

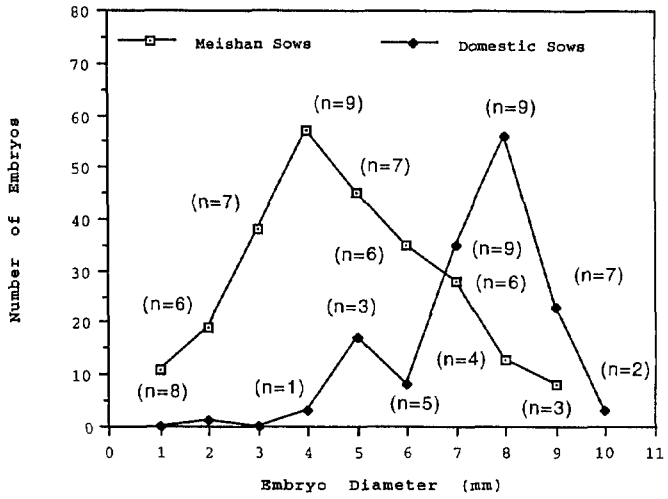

Figure 1. Distribution of embryonic diameter for embryos recovered on d 11.9 from Meishan (n = 11) and domestic (n = 10) sows. The numbers in parentheses indicate the number of sows that donated one or more embryos to each data point.

Table 3. Mean DNA content and protein/DNA ratio from embryos from Meishan and domestic sows

Breed	n	DNA content, μg/embryo	Protein/DNA
Meishan	11	2.4 ± .4[a]	26.2 ± 3.3[a]
Domestic	10	4.7 ± .5[b]	22.9 ± 1.4[a]

[a,b]Means ± SEM within a column with different superscripts differ ($P < .001$).

In addition, the $E_2\beta$ content in uterine flushings was lower ($P < .01$) in Meishan than in domestic sows.

Because breed differences in DNA content and $E_2\beta$ content were observed, the data for embryos in each breed were then pooled across sows according to embryo diameter in the following increments: < 1.0, 1.0 to 1.9, 2.0 to 2.9, 3.0 to 3.9, 4.0 to 4.9, 5.0 to 5.9, 6.0 to 6.9, 7.0 to 7.9, 8.0 to 8.9, and 9.0 to 9.9 mm. Embryonic DNA content increased ($P < .001$) with increasing embryonic diameter, whereas the protein: DNA ratio remained constant in blastocysts that ranged from 1 to 10 mm. Nonsignificant ($P > .10$) breed × size interactions indicated that embryos of the same size from both breeds contained the same number of cells of similar size.

No difference ($P > .10$) in embryonic $E_2\beta$ content was observed between breeds for embryos < 5 mm in diameter (Figure 2). However, as embryonic diameter increased beyond 6 mm, embryos from Meishan sows contained less ($P < .001$) $E_2\beta$ than did embryos from domestic sows. This resulted in a highly significant ($P < .001$) breed × diameter interaction. Regression analysis revealed that the pattern of $E_2\beta$ production by Meishan embryos was linear ($Y = -288.3 + 120.0X$; $P < .01$), whereas that of domestic embryos was described as a second-order polynomial ($Y = 15.04 - 30.80X + 20.45X^2$; $P < .05$).

Discussion

Two factors that may affect litter size, ovulation rate and early embryonic diversity, were examined in this experiment. Meishan sows had a markedly higher ovulation rate and a correspondingly greater number of embryos than did domestic sows. Comparison of ovulation rate in Meishan gilts and gilts of European breeds have been inconsistent (Bolet et al., 1986; Bazer et al., 1988; Haley et al., 1990). In these studies, the inconsistency in ovulation rate data can be explained by the age of the gilts at the time these data were collected. Ovulation rate and litter size have been reported to increase with age in both domestic (Robertson et al., 1951; Kirkwood and Aherne, 1985) and Meishan (Cheng, 1983; Legault, 1985) gilts, although the rate of increase seems to be markedly greater in Meishan gilts (Cheng, 1983; Legault, 1985). The ovulation rate increase is approximately twofold greater from the first to the sixth estrous cycle in Meishan than in domestic crossbred gilts (R. K. Christenson, 1992, unpublished data).

The greater number of embryos recovered from Meishan sows than from domestic sows coincides well with litter size data collected previously at Clay Center. These second-parity Meishan sows produced 4.5 more pigs per litter than did domestic sows, which averaged 9.0 pigs farrowed per litter (R. K. Christenson, 1992, unpublished data).

The death of less-developed littermate embryos in pigs may result from their asynchrony with the uterine environment (Pope et al., 1982; Wilde et al., 1988), because these less-developed embryos are more likely to die after estrogen-induced alterations in uterine histotroph composition (Morgan et al., 1987). Embryonic estrogen production increases with increasing embryonic development (Ford et al., 1982;

Table 4. Embryonic and uterine luminal estrogen contents from Meishan and domestic sows

Breed	n	Embryonic $E_2\beta$ content, pg/embryo	Uterine flushings $E_2\beta$ content, pg/mL
Meishan	11	221.3 ± 59.1[a]	61.0 ± 18.2[c]
Domestic	10	699.2 ± 105.1[b]	163.7 ± 32.0[d]

[a,b]Means ± SEM within a column with different superscripts differ ($P < .001$).

[c,d]Means ± SEM within a column with different superscripts differ ($P < .01$).

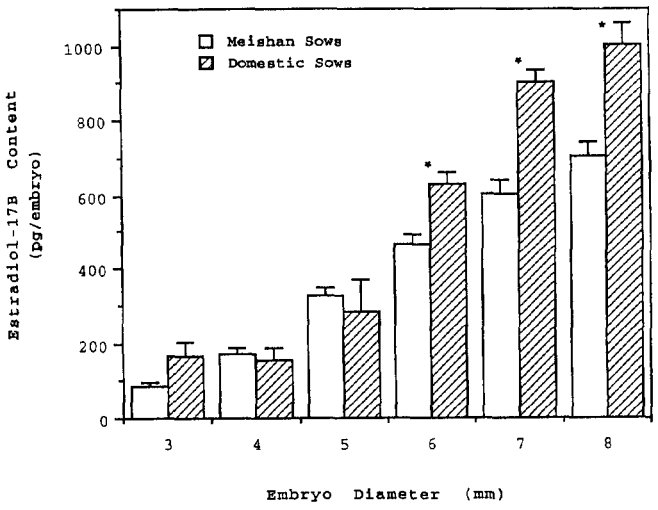

Figure 2. Estradiol-17β content of embryos of different diameters (millimeters) collected on d 11.9 from both Meishan (n = 11) and domestic (n = 10) sows. Each bar represents the mean ± SEM of embryos of a particular diameter from each breed. Asterisks denote differences ($P < .001$) between breeds for that embryonic diameter. Differences between diameters were determined using Student *t*-tests.

Pusateri et al., 1990) and synchronizes the release of secretory granules by the uterine glandular epithelium (Geisert et al., 1982). Results from the present study indicate that the more-advanced littermate embryos from Meishan sows produce less $E_2\beta$ than do embryos of the same size from domestic sows. Therefore, the lower rate of $E_2\beta$ production by the more-developed embryos in a Meishan litter may alter the uterine environment more gradually, increasing the probability of survival of less-developed littermates.

In contrast to previous reports (Bazer et al., 1988; Wilmut et al., 1992), embryos from Meishan sows were smaller on d 12 of gestation than were embryos from domestic sows. The smaller embryonic size may have resulted from either a difference in the time of ovulation or an inherent reduction in embryonic developmental rate dictated by either embryonic genes or maternal genes controlling uterine/embryo interactions.

Meishan sows have been reported to ovulate approximately 15 h later than Large White sows (Wilmut et al., 1992). Although not directly investigated, data from the present experiment suggest little difference in the interval from the onset of estrus to ovulation between Meishan and domestic sows. Previous research has associated longer lengths of estrus with a delayed time of ovulation (Burger, 1952). No difference in the length of estrus was observed in the present experiment between Meishan (2.5 ± .2 d) and domestic (2.4 ± .1 d) sows. Further, the range in embryo size was not different between breeds (Figure 1), again suggesting little difference in the time of ovulation between breeds.

Recent data from Iowa State University also suggest small, if any, breed difference in the time of ovulation between Meishan and domestic (Yorkshire) gilts. No difference was observed in the percentage of Meishan and Yorkshire gilts that had completed ovulation or were in the process of ovulation from 48 to 54 h after the first detection of estrus (Youngs et al., 1992). Further, the embryos collected from these gilts had the same range in cell numbers (one to eight cells).

As previously stated, Meishan embryos were smaller on d 12 than embryos from domestic sows. This suggests that embryos from Meishan sows may exhibit a reduced developmental rate. In support of this hypothesis, Youngs et al. (1993) observed that embryos from Meishan gilts developed more slowly in vitro than did embryos from domestic (Yorkshire) gilts. Previous research has associated differences in embryonic genotype with differences in both preimplantation embryonic developmental rate and litter size in miniature pigs (Ford et al., 1988) and mice (Goldbard and Warner, 1982). Collectively, these data suggest that the reduced developmental rate observed for embryos from Meishan sows may be controlled by embryonic genes and may influence embryonic mortality.

Maternal genes may also affect embryonic development and may control the increased prenatal survival in Meishan sows (Haley et al., 1990). Components in uterine histotroph have been reported to affect embryonic development (Roberts and Bazer, 1988). Temporal differences in embryonic development and uterine histotroph composition have been observed between Meishan and Large White gilts (Bazer et al., 1988, 1991; Simmen et al., 1989). The discrepancy between the findings of the present experiment and these previous reports may have resulted from the method of determining onset of estrus (four times daily in our study vs two times daily by other groups) or from the difference in the genetic background of the Meishan pigs. Our Meishans were genetically diverse, representing 8 to 10 family lines. The original study by Bazer et al. (1988) used Meishan gilts derived from littermate matings of two Meishan gilts and a single Meishan boar (Bidanel et al., 1990). Therefore, the data obtained by Bazer and coworkers (1988) may not be as representative of the Meishan population.

We conclude that the prolificacy of the Meishan sow cannot be attributed to an increase in embryonic developmental synchrony. Increased litter size of the Meishan sow may result from a coupling of an increased ovulation rate, a reduced embryonic developmental rate, and the reduced production of $E_2\beta$ by the developing embryos.

Implications

One method of investigating the factors that control litter size in swine is to determine and characterize differences between prolific breeds (Meishan) and breeds with average litter sizes. Data obtained in this study indicate that differences exist in both embryonic growth rate and steroid secretory rate between breeds with differing litter sizes. The reduced growth rate and estrogen production by embryos from Meishan sows may result in more gradual alteration in uterine environment, thereby increasing the chance of survival of the less-developed littermate embryos.

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